

Identification of Patients at Low Risk for Recurrent Venous Thromboembolism by Measuring Thrombin Generation

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ANTICOAGULANT TREATMENT for patients with venous thromboembolism (VTE) consists of low-molecular-weight heparin with therapeutic doses followed by vitamin K antagonists for at least 3 to 6 months.¹ After discontinuation of anticoagulant treatment, a third of patients experience recurrence of VTE within the next 5 to 8 years.² The case-fatality rate of recurrence is about 5%.³ Therefore, identification of patients who might benefit from indefinite anticoagulant treatment (ie, patients in whom recurrent VTE is more likely than anticoagulation-associated severe bleeding) is now one of the foremost goals in thrombosis research.

The likelihood of recurrent VTE varies within individuals and is strongly influenced by the presence of persistent and/or acquired risk factors. Among the multiple risk factors for thrombosis, deficiencies of antithrombin, protein C, or protein S; homozygous or combined defects' antiphospholipid antibodies; and high plasma level of factor VIII appear to be relevant for recurrent disease.⁴ Many patients with VTE,

Context Screening of patients with venous thromboembolism (VTE) for thrombophilic risk factors is common clinical practice. Because of the large number of risk factors, assessing the risk of recurrence in an individual patient is complex. A method covering multicausal thrombophilia is therefore required.

Objective To investigate the relationship between recurrence of VTE and a simple global coagulation assay measuring thrombin generation.

Design, Setting, and Participants Prospective cohort study of 914 patients with first spontaneous VTE who were followed up for an average of 47 months after discontinuation of vitamin K antagonist therapy. The study was conducted at the Department of Internal Medicine I, Medical University of Vienna, Vienna, Austria, between July 1992 and July 2005. Thrombin generation was measured by a commercially available assay system. Patients with a previous or secondary VTE; antithrombin, protein C, or protein S deficiencies; presence of lupus anticoagulant; cancer; or pregnancy were excluded.

Main Outcome Measure Objectively documented symptomatic recurrent VTE.

Results Venous thromboembolism recurred in 100 patients (11%). Patients without recurrent VTE had lower thrombin generation than patients with recurrence (mean [SD], 349.2 [108.0] nM vs 419.5 [110.5] nM, respectively; $P < .001$). Compared with patients who had thrombin generation greater than 400 nM, the relative risk (RR) of recurrence was 0.42 (95% confidence interval [CI], 0.26-0.67; $P < .001$) in patients with values between 400 nM and 300 nM; for patients with lower values, the RR was 0.37 (95% CI, 0.21-0.66; $P = .001$). After 4 years, the probability of recurrence was 6.5% (95% CI, 4.0%-8.9%) among patients with thrombin generation less than 400 nM compared with 20.0% (95% CI, 14.9%-25.1%) among patients with higher values ($P < .001$). Patients with thrombin generation less than 400 nM, representing two thirds of patients, had a 60% lower RR of recurrence than those with greater values (RR, 0.40; 95% CI, 0.27-0.60; $P < .001$).

Conclusion Measurement of thrombin generation identifies patients at low risk for recurrent VTE.

JAMA. 2006;296:397-402

www.jama.com

however, have more than 1 thrombophilic condition, and important risk factors for VTE are hitherto unidentified. Ideally, a single laboratory test that would detect multifactorial thrombophilia could help determine the overall risk of recurrent VTE.

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See also Patient Page.

Generation of thrombin is the pivotal step of hemostasis. According to *in vitro* experiments, thrombin generation occurs in 2 phases. Initially, small amounts of thrombin are produced after the activation of factor X by tissue factor/factor VIIa. The initial thrombin propagates coagulation by activating platelets, factor V, and factor VIII. Factors VIIIa and IXa, which are generated by tissue factor/factor VIIa, combine on the surface of activated platelets leading to factor X activation. This results in the generation of large amounts of thrombin, fibrin formation, and, ultimately, clot formation.⁵

Thrombin activity can be registered by continuously measuring cleavage of a chromogenic or fluorescent substrate, resulting in a thrombin generation curve. From this curve, various parameters, including time until thrombin burst, peak amount of thrombin generation, velocity of thrombin generation, or total amount of thrombin generated (area under the thrombin generation curve), can be inferred. In a study by Dargaud et al,⁶ reduced thrombin generation was recorded in patients with bleeding tendency, such as patients with hemophilia A or B. Conversely, thrombin generation is increased in patients at risk for VTE, such as women who take oral contraceptives⁷ or patients with natural inhibitor deficiencies^{8,9} or factor II G20210A.¹⁰ Using a numerical simulation model, Brummel-Ziedins et al¹¹ recently showed that thrombin generation based on the individual's blood composition is associated with risk of first venous thrombosis.

Therefore, we hypothesized that by measurement of thrombin generation, patients with VTE could be stratified into high- and low-risk categories for recurrence. To test this hypothesis, we followed 914 patients with a first spontaneous VTE and assessed the relationship between thrombin generation and risk of recurrence.

METHODS

Patients and Study Design

The Austrian Study on Recurrent Venous Thromboembolism (AUREC) is

an ongoing, prospective cohort study involving patients from 4 thrombosis centers in Vienna, Austria.¹² The ethics committee of the Medical University of Vienna approved the study, and written informed consent was obtained from all patients at the beginning of the study. Between July 1992 and July 2005, 2977 consecutive patients older than 18 years who had been treated for at least 3 months with vitamin K antagonists after an unprovoked episode of VTE were enrolled. All patients were treated with standard heparin to keep the activated partial thromboplastin time at 1.5 to 2 times the control value or received subcutaneous therapeutic doses of low-molecular-weight heparin.

Of 2977 patients enrolled, 2063 were excluded for the following reasons: surgery, trauma, or pregnancy within the previous 3 months (462); cancer (458); or need for indefinite treatment with antithrombotic drugs for reasons other than VTE (453). A total of 377 patients with more than 1 episode of VTE; 75 patients with the lupus anticoagulant; and 72 patients with antithrombin, protein C, or protein S deficiencies received indefinite anticoagulant therapy with vitamin K antagonists and were therefore excluded. We also excluded 140 patients in whom material for laboratory testing was not available, and 26 patients with factor VIII levels greater than 230 IU/dL who were included in a prospective trial investigating the effect of long-term anticoagulant therapy.

Patients were enrolled at the time of discontinuation of vitamin K antagonist therapy, ie, at least 3 months after acute venous thrombosis. For measurement of antithrombin, protein C, protein S, the lupus anticoagulant, factor V Leiden, factor II G20210A, and factors VIII and IX, venous blood was collected 3 weeks after discontinuation of vitamin K antagonists. For measurement of peak thrombin generation, blood was collected at a median of 13 months (3 weeks to 94 months) after discontinuation of anticoagulant therapy. Patients were seen at 3-month

intervals during the first year and every 6 months thereafter. They were given detailed written information on symptoms of VTE and were asked to report immediately to one of the participating centers if such symptoms occurred. A medical history was obtained and a physical examination was performed at each visit. Female patients were strongly discouraged from taking oral contraceptives or undergoing hormone replacement therapy.

Diagnosis of VTE

Criteria for diagnosis of VTE have been reported in detail.¹² Deep vein thrombosis was confirmed by venography or color-coded duplex sonography (in proximal deep vein thrombosis only). Venography had to meet at least 1 of the following criteria: a constant filling defect present on 2 views; an abrupt discontinuation of the contrast-filled vessel at a constant point in the vein; or failure of the entire deep-vein system to fill without an external compressing process, with or without venous flow through collateral veins. For color-coded duplex sonography, at least 1 of the following criteria had to be met: visualization of an intraluminal thrombus in a deep vein, lack of compressibility, or incomplete compressibility.

Diagnosis of pulmonary embolism was established by a positive finding on ventilation-perfusion lung scanning according to the criteria of the Prospective Investigation of Pulmonary Embolism Diagnosis¹³ or by spiral computed tomography displaying 1 or several low-attenuation areas that partly or completely filled the lumen of an opacified vessel. Patients with both deep vein thrombosis and pulmonary embolism were classified as having pulmonary embolism.

Outcome

The end point of the study was recurrent symptomatic deep vein thrombosis or recurrent symptomatic pulmonary embolism diagnosed according to the aforementioned criteria. Recurrence of deep vein thrombosis was

diagnosed if the patient had (1) a thrombus in the leg not involved in the previous event; (2) another vein in the leg involved in the previous event; or (3) a thrombus in the same venous system involved in the previous event with a proximal extension of the thrombus if the upper limit of the original thrombus had been visible, or the presence of a contrast filling defect surrounded by contrast medium if it had not.

Laboratory Analysis

Venous blood was collected into 0.1 volume of 3.6% trisodium citrate, centrifuged for 20 minutes at 2000 g, and stored at -80°C until time of analysis. Thrombin generation was determined using an assay kit (Technothrombin TGA, Technoclone, Vienna, Austria) on a fully automated, computer-controlled microplate reader (Genios, Tecan, Männedorf, Switzerland) and specially adapted software (Technothrombin TGA). In this assay kit, thrombin generation is initiated in citrated plasma by 71.6 pM recombinant human tissue factor lipidated in 3.2 μM phospholipid micells (phosphatidylcholine [2.56 μM] and phosphatidylserine [0.64 μM]). For analysis, the maximum concentration of thrombin (peak thrombin) generated was used. Antithrombin, protein C, and protein S values were determined according to routine laboratory methods. The presence of the lupus anticoagulant was assessed according to the criteria of the International Society of Thrombosis and Haemostasis.¹⁴ Screening for factor V Leiden and for factor II G20210A was carried out as described.^{15,16} Factor VIII and factor IX were measured by 1-stage clotting assays as recently described.^{17,18}

Statistical Analysis

Times to recurrence (uncensored observations) or follow-up times in patients without recurrence (censored observations) were analyzed using survival time methods.¹⁹ The probability of recurrence was estimated according to the Kaplan-Meier method.²⁰ To test for homogeneity between strata, we applied

the log-rank test. Categorical data were checked for homogeneity using contingency table analyses (χ^2 test), and the Mann-Whitney test was used for continuous data. Univariate and multivariate Cox proportional hazard models were used to analyze the association of risk for recurrent VTE and peak generation of thrombin. Adjustments included sex, age, body mass index, duration of anticoagulant therapy, location of first event, factor V Leiden, and factor II G20210A. All data are given as mean (SD) unless otherwise indicated, and $P < .05$ is considered statistically significant. SPSS version 12.0 (SPSS Inc, Chicago, Ill) was used for all statistical computations.

RESULTS

Patients

Baseline characteristics of the 914 patients are shown in TABLE 1. One hundred ninety-four patients were excluded during the course of the study due to diagnosis of cancer (17), requirement of antithrombotic drugs for reasons other than VTE (such as arterial disease or atrial fibrillation) (119), pregnancy (34), or loss to follow-up (12). Twelve patients died for reasons other than VTE: cardiac failure (6), cancer (2), choking asphyxia (1), cerebral hemorrhage (1), aortic dissection (1), and suicide (1). Patients were followed up until the time of exclusion or death, when the data were censored.

Recurrence of VTE

Venous thromboembolism recurred in 100 patients (11%) (deep vein thrombosis in 58 patients and pulmonary embolism in 42 patients). Pulmonary embolism was fatal in 3 patients. Recurrence was spontaneous in 90 patients and secondary to surgery or trauma in 10 patients. Recurrence was seen in 69 men and 31 women. Patients with recurrent VTE had higher concentrations of factor VIII (173 [45] IU/dL vs 165 [45] IU/dL; $P = .15$) and factor IX (127 [29] IU/dL vs 118 [25] IU/dL; $P = .002$), had a shorter observation period (32 vs 48 months;

Table 1. Baseline Characteristics of Patients (N = 914)*

Characteristic	Value
Age at first VTE, mean (SD), y	47 (16)
Sex	
Male	408 (45)
Female	506 (55)
Site of thrombosis	
Deep vein thrombosis	491 (54)
Pulmonary embolism	423 (46)
Factor V Leiden	
Heterozygote	242 (27)
Homozygote	18 (2)
Prothrombin G20210A	63 (7)
Factor VIII, mean (SD), IU/dL	166 (45)
Factor IX, mean (SD), IU/dL	119 (26)
Duration of oral anticoagulant therapy, mean (SD), mo	8 (11)
Observation time, mean (SD), mo	47 (37)

Abbreviation: VTE, venous thromboembolism.

*Data are expressed as No. (%) unless otherwise indicated.

$P < .001$), and were older (50 vs 47 years; $P = .08$). Factor V Leiden was detected in 38 patients with recurrence (38%) and in 222 patients (27%; $P = .03$) without recurrence. Factor II G20210A was found in 9 patients with recurrence (9%) and in 54 patients (7%; $P = .38$) without recurrence.

Peak Thrombin Generation and Risk of Recurrent VTE

Women had higher peak thrombin generation than men (366.5 [108.6] nM vs 345.0 [111.6] nM; $P = .009$), and carriers of factor II G20210A had higher peak thrombin generation than patients without the mutation (396.1 [122.4] nM vs 353.6 [108.9] nM; $P = .004$). No difference in peak thrombin generation was seen between patients with and without factor V Leiden (357.5 [109.5] nM vs 356.1 [110.7] nM; $P = .90$) or between patients with and without high factor VIII (370.3 [106.3] nM vs 356.1 [110.7] nM; $P = .43$). A second measurement of peak thrombin generation was performed in a randomly selected subset of 319 patients. The predefined time interval between first and second measurement was at least 12 months and not more than 30 months. No significant difference between first and second peak thrombin measurement was found ($P = .67$). The correlation coefficient between the 2 measurements was .46 ($P < .001$).

Table 2. Relative Risk of Recurrent Venous Thromboembolism According to Tertiles

Peak Thrombin, nM	No. of Patients	No. of Recurrences	RR (95% CI)	
			Univariate	Multivariate*
≥400	305	61	1	1
300-400	319	24	0.42 (0.26-0.67)	0.45 (0.28-0.73)
<300	290	15	0.37 (0.21-0.66)	0.37 (0.20-0.67)

Abbreviations: CI, confidence interval; RR, relative risk.

*Adjusted for age, sex, body mass index, location of first thrombosis, duration of oral anticoagulant therapy, factor V Leiden, and factor II G20210A.

Table 3. Characteristics of Patients According to Peak Thrombin Generation*

Characteristic	Peak Thrombin Generation <400 nM (n = 609)	Peak Thrombin Generation ≥400 nM (n = 305)	P Value
Age at first VTE, mean (SD), y	46 (16)	49 (14)	.003
Men	281 (46)	127 (42)	.20
Site of thrombosis			
Deep vein thrombosis	313 (51)	178 (58)	.05
Pulmonary embolism	296 (49)	127 (42)	
Factor V Leiden	174 (29)	86 (28)	.94
Factor II G20210A	31 (5)	32 (11)	.002
High factor VIII†	53 (9)	36 (12)	.14
High factor IX‡	127 (21)	105 (34)	<.001

Abbreviation: VTE, venous thromboembolism.

*Data are expressed as No. (%) unless otherwise indicated.

†High factor VIII was defined as factor VIII level greater than 221 IU/dL (90th percentile of patient cohort).

‡High factor IX was defined as factor IX level equal to or greater than 133 IU/dL (75th percentile of patient cohort).

Patients without recurrent VTE had significantly lower peak thrombin generation than patients with recurrence (349.2 [108.0] nM vs 419.5 [110.5] nM; $P<.001$). No difference was observed between patients with spontaneous recurrent venous thrombosis and patients with recurrence secondary to a precipitating factor (419.3 [108.5] nM vs 421.5 [133.8] nM; $P=.92$).

When peak thrombin generation was analyzed as a continuous variable in a Cox proportional hazard model, the relative risk (RR) of recurrence was 1.04 (95% confidence interval [CI], 1.02-1.06; $P<.001$) for each 10-nM increase and remained identical after adjustment for age, sex, body mass index, location of first thrombosis, duration of oral anticoagulant therapy, factor V Leiden, and factor II G20210A.

The RR of recurrent VTE associated with each of several different ranges of peak thrombin generation is shown in TABLE 2. Compared with patients who had peak thrombin generation of 400 nM or greater, the RR of recurrence was 0.42 (95% CI, 0.26-0.67; $P<.001$)

in patients with values between 400 nM and 300 nM; for patients with values less than 300 nM, the RR was 0.37 (95% CI, 0.21-0.66; $P=.001$). Similar results were obtained in the multivariate analysis.

We next compared the risk of recurrent VTE between patients who had peak thrombin generation equal to or greater than 400 nM with patients who had peak thrombin generation of less than 400 nM. The cutoff of 400 nM was chosen on the basis of Cox regression analyses of RRs based on tertiles. The lower limit of the upper tertile was 400 nM and was the best discriminator between low- and high-risk patients. Patients with peak thrombin generation of less than 400 nM were younger and a larger proportion had low levels of factor VIII or IX. No difference between the 2 groups was found with respect to factor V Leiden (TABLE 3). At 4 years, the probability of recurrent VTE was 6.5% (95% CI, 4.0%-8.9%) in patients who had peak thrombin generation less than 400 nM compared with 20.0% (95% CI, 14.9%-25.1%) in patients who

had peak thrombin generation equal to or greater than 400 nM ($P<.001$ by log-rank test [FIGURE]). Compared with patients who had peak thrombin generation equal to or greater than 400 nM, the RR of recurrence was 0.40 (95% CI, 0.27-0.60; $P<.001$) in those with a lower value. The RR did not substantially change after adjustment for age, sex, body mass index, location of first thrombosis, duration of oral anticoagulant therapy, factor V Leiden, and factor II G20210A (RR, 0.42; 95% CI, 0.27-0.63; $P<.001$).

The risk of recurrent VTE was estimated in patients in whom peak thrombin generation was measured in blood obtained earlier than 13 months after discontinuation of anticoagulant therapy compared with patients in whom peak thrombin generation was measured in samples obtained at a later time point. The corresponding RRs for patients with peak thrombin generation less than 400 nM adjusted for the above variables were almost identical (0.33; 95% CI, 0.19-0.57; $P=.002$ vs 0.34; 95% CI, 0.17-0.67; $P<.001$).

COMMENT

In this large prospective cohort study, we found that patients with a first spontaneous VTE and peak thrombin generation of less than 400 nM after discontinuation of vitamin K antagonists have a low risk of recurrence. According to Kaplan-Meier analysis, the likelihood of recurrent VTE in these patients was as low as 7% after 4 years, with an upper 95% CI of 9%. Compared with patients who had higher levels, those with peak thrombin generation less than 400 nM had an almost 60% lower risk of recurrence. Most importantly, the group of patients with low peak thrombin generation represented two thirds of the total patient population.

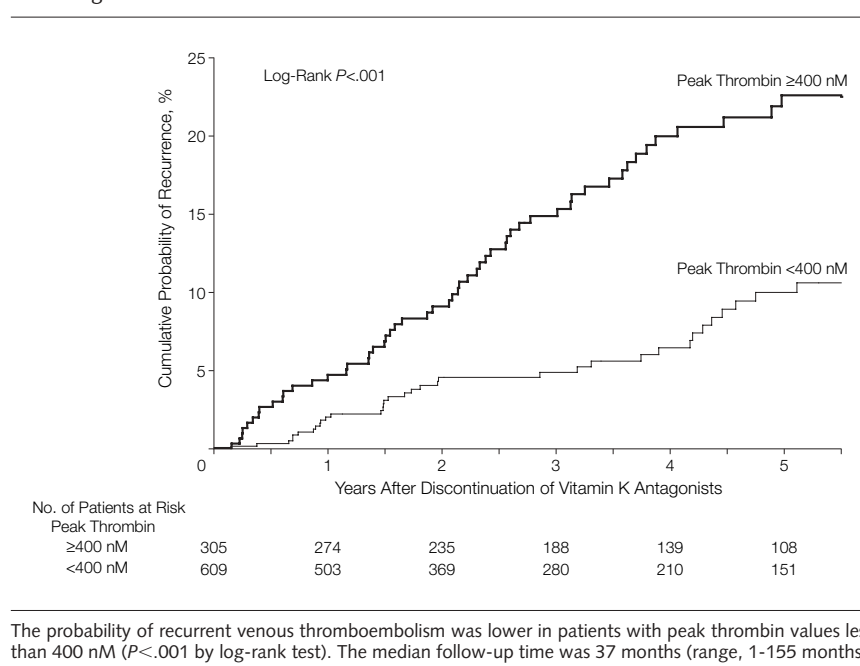
The optimal duration of secondary thromboprophylaxis following VTE is unclear. Vitamin K antagonists provide an almost complete protection against VTE but have the disadvantage of patient inconvenience and risk of

bleeding. The incidence rates of major hemorrhages among patients treated with vitamin K antagonists at an international normalized ratio of 2 to 3 range between 2 and 4 per 100 patient-years,^{21,22} with incidence rates of intracranial hemorrhage between 0.1 and 0.3 per 100 patient-years.²³ Expanding vitamin K antagonist therapy beyond 3 to 6 months is thus only justified in patients in whom the risk of bleeding is outweighed by the likelihood of recurrent VTE. How to identify these patients is, however, still an unexplained puzzle. Over the years, several laboratory risk factors for recurrent VTE have been defined and, as a consequence, extensive routine thrombophilia screening has become common practice. Thrombophilia screening is costly and often inconclusive, as the impact of some defects on the recurrence risk is uncertain, many patients have more than 1 risk factor, and hitherto unidentified risk factors exist.

In this context, we believe that our findings are of major clinical relevance. Using a simple commercially available laboratory method developed to measure thrombin generation, we were able to identify patients in whom the long-term risk of recurrent VTE is almost negligible. Considering the incidence rates of severe or fatal hemorrhage related to anticoagulant therapy and the case-fatality rate of recurrent VTE, patients with low peak thrombin generation (<400 nM) would almost certainly not benefit from indefinite anticoagulant therapy. Consequently, extensive thrombophilia screening appears to be unnecessary in this large, low-risk patient group. In this respect, it is of interest that important thrombotic risk factors including high plasma levels of factor VIII and factor IX or factor II *G20210A* were less prevalent in patients with peak thrombin generation less than 400 nM compared with patients who had higher levels.

Some limitations of this study need to be addressed. Our data cannot be applied to several patient groups. Patients with antithrombin, protein C, or

Figure. Kaplan-Meier Method Estimates of the Risk of Recurrent Venous Thromboembolism According to Peak Thrombin Generation



The probability of recurrent venous thromboembolism was lower in patients with peak thrombin values less than 400 nM ($P < .001$ by log-rank test). The median follow-up time was 37 months (range, 1-155 months).

protein S deficiencies were regarded as candidates for indefinite anticoagulant therapy and, hence, excluded from the study. There is, however, evidence in the literature that thrombin generation is increased not only in antithrombin-deficient patients, but also in patients with low protein C or S plasma levels.^{8,9,24} Thus, patients with a natural coagulation inhibitor deficiency in whom the risk for recurrence appears to be high will most likely exhibit high peak thrombin generation. In our institution, patients with more than 1 episode of VTE and patients with VTE secondary to the lupus anticoagulant routinely receive indefinite anticoagulant treatment and were therefore excluded from the study. We also excluded low-risk patients with VTE secondary to surgery, trauma, or pregnancy, in whom extended anticoagulant treatment is usually not justified. The median interval between discontinuation of anticoagulant treatment and measurement of peak thrombin generation was 13 months and varied from patient to patient. To investigate the influence of differences in the time

of measurement, we determined peak thrombin generation in a second sample. The 2 measurements significantly correlated and no statistical difference was found between first and second peak thrombin measurement. Furthermore, the risk of recurrence was identical in patients in whom peak thrombin generation was determined in samples obtained earlier and later than 13 months after cessation of anticoagulant treatment, respectively. These findings suggest persistence of peak thrombin generation measurements over time. AUREC is a hypothesis-generating cohort study, which precludes predefinition of certain cutoff values. Therefore, our observation should serve as the basis for validation in a prospective interventional outcome trial.

CONCLUSION

Using a simple and inexpensive laboratory assay system to measure thrombin generation, we were able to identify a large number of thrombosis patients with a low risk of recurrence. These patients represent two thirds of our cohort and will most likely not ben-

efit from indefinite treatment with vitamin K antagonists.

Author Contributions: Dr Kyrle had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Eichinger, Kyrle.

Acquisition of data: Kollars.

Analysis and interpretation of data: Hron, Binder, Eichinger, Kyrle.

Drafting of the manuscript: Hron, Kyrle.

Critical revision of the manuscript for important intellectual content: Kollars, Binder, Eichinger.

Statistical analysis: Hron.

Obtained funding: Eichinger, Kyrle.

Administrative, technical, or material support: Kollars, Binder.

Study supervision: Eichinger.

Financial Disclosures: Dr Binder has reported that he is chief scientific officer for Technoclone GmbH, Vienna, Austria.

Funding/Support: This work was supported by grants from the Medizinisch-Wissenschaftlicher Fonds des Bürgermeisters der Bundeshauptstadt Wien and the Jubiläumsfond of the Austrian National Bank. The Technothrombin TGA assay kit and the respective equipment necessary for analysis were provided by Technoclone, Vienna, Austria.

Role of the Sponsor: The sponsors had no involvement in the design and conduct of the study; in the collection, management, analysis, and interpretation of the data; or in the preparation of the manuscript.

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